Intervirology
Editor-in-Chief: F. Rapp, Hershey, Pa.

Reprint
Publisher: S. Karger AG, Basel
Printed in Switzerland

Intervirology 29: 133–143 (1988)

0300–5526/88/0293/0133$2.75/0

Viral Strain Dependent Differences in Experimental Argentine Hemorrhagic Fever (Junin Virus) Infection of Guinea Pigs

Richard H. Kenyon[a], *D.E. Green*[a], *J.I. Maiztegui*[b], *C.J. Peters*[a]

[a] Disease Assessment Division and Pathology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Md., USA;
[b] Instituto Nacional de Estudios Sobre Virosis Hemorragicas, Pergamino, Province de Buenos Aires, Argentina

Key Words. Argentine hemorrhagic fever virus · Junin virus · Arenavirus

Summary. Guinea pigs infected with low-passage Junin virus of human origin showed viral strain dependent differences in mortality, LD_{50}, time to death, and in viral spread and distribution. Different Junin strains appeared to cause at least two broad patterns of Argentine hemorrhagic fever in guinea pigs. A number of strains of Junin virus caused a viscerotropic type of illness in which virus replicated predominantly in lymph nodes, spleen, and bone marrow. With the most severe visceral forms of Argentine hemorrhagic fever, the guinea pigs became viremic, developed necrosis of spleen, lymph nodes, and bone marrow, showed gastric hemorrhages, and all animals died within 13–15 days. Other Junin strains induced a neurological type of illness with transient viral replication in and lymphocyte depletion of spleen and lymph nodes, with no detectable viremia or viral replication in bone marrow. Subsequently, virus was found in the brain with varying severities of polioencephalitis, and the guinea pigs frequently showed rear leg paralysis before death occurred 28–34 days after inoculation. Not all animals infected with a neurotropic strain developed all these signs. One viral strain induced some signs characteristic of both patterns of illness. Although the disease forms in the guinea pig model did not strictly correlate with those observed in the humans from which these strains were obtained, the different strains of Junin virus consistently caused very different patterns of illness in infected guinea pigs.

Address inquiries to: Dr. Richard H. Kenyon, Disease Assessment Division, USAMRIID, Fort Detrick, Frederick, MD 21701-5011 (USA)

Received: November 16, 1987
Accepted: February 4, 1988

Introduction

Junin virus causes Argentine hemorrhagic fever (AHF), an acute, severe disease with mortality rates of 15–30% in untreated cases. The reservoir for Junin virus is *Calomys musculinus*, a small rodent inhabiting corn and

soybean fields of Argentina. Human infection is believed to occur predominantly by aerosol exposure. Our current knowledge of AHF pathogenesis has been enhanced by data from the experimental infection of mice [1, 2], monkeys [3–5], and, especially, guinea pigs [6, 7]. Although valuable information has been gathered by using mice, the experimental disease in this model is dissimilar from that seen in man. Several New World and Old World monkeys have been evaluated as prospective models for AHF. Rhesus macaques were established as a model closely reflecting the clinical features of AHF in humans [4, 5]. Disadvantages of this model, however, are cost, scarcity, and the large size of the animals, restricting their utility to very specialized studies. Much of the current knowledge of AHF pathogenesis has been derived from studies with guinea pigs, and most of these have been conducted with a single strain of Junin virus, the prototype XJ strain [6, 8].

In humans, a spectrum of clinical AHF disease patterns is observed. Some individuals are only mildly ill, whereas others show a severe febrile and hemorrhagic illness. Experienced physicians generally classify AHF into mild or common, hemorrhagic, neurological, and mixed forms [9, 10]. The purpose of the experiments presented here was twofold: (i) to determine whether low passage level Junin virus from confirmed human AHF cases caused a consistently lethal disease pattern in guinea pigs and (ii) to determine whether the patterns of illness produced by the different viral strains in guinea pigs correlated with the pattern of illness that these same strains caused in humans.

Materials and Methods

Viral Strains

The attenuated Junin viral strain, designated XJ44, was obtained by 44 successive suckling mouse

Table I. Virulence of different Junin viral strains in guinea pigs

Strain designation[a]	Human clinical disease type	Number dead/ total[b]	Number paralyzed/ total	$\log_{10}$ PFU/ LD_{50}	MTD to days[c]
P3790	fatal hemorrhagic	20/20	0/20	-1.3	17.3 ± 2.7
P3235	nonfatal mixed	20/20	0/20	-0.7	14.5 ± 1.7
P3406	fatal neurological	16/18	0/18	-0.5	19.0 ± 2.3
P3551	fatal mixed	11/15	1/15	2.2	21.1 ± 2.5
P3684	nonfatal mild	4/10	3/10	4.0	27.8 ± 3.9
P3827	fatal mixed	4/20	4/20	-	28.0 ± 5.9
XJ44	-	0/20	0/20	-	none
Candid No. 1	-	0/20	0/20	-	none

[a] All strains except XJ44 and Candid No. 1 were isolated from patients with AHF. XJ44 is a laboratory-derived, attenuated strain. Candid No. 1 is an AHF vaccine candidate.

[b] There were 5,000 PFU/inoculum.

[c] For MTD (± SD), guinea pigs were inoculated intraperitoneally with 100 LD_{50} of all strains except P3827. Animals inoculated with P3827 received 5.0 $\log_{10}$ PFU.

brain passages of the virulent XJ strain of Junin virus [11]. Candid No. 1, another attenuated strain, was derived by cloning and passage of XJ44 and is currently being tested as a human vaccine [7]. All other strains were obtained from patients with confirmed AHF and were from the endemic areas of Argentina. Other publications have referred to some of these stains as Romero (P3235), Coronel (P3827), Espindola (P3790), and Ledesma (P3406) strains. Blood samples from ill patients (strain P3551, postmortem liver tissue) were inoculated onto MRC-5 human diploid lung cell monolayers. Viruses were passaged an additional time in these cells and then once in Vero cells. Dilutions of virus were made in L-15 medium supplemented with heat-inactivated fetal bovine serum (2%). Infectious virus was quantitated by counting plaques in Vero cells, as described by Webb et al. [12]. All viral strains, except Candid No. 1 and XJ44, were manipulated under P-4 containment [13, 14].

Guinea Pigs

We used male Hartley strain outbred guinea pigs (350–400 g) obtained from Charles River Laboratories and inoculated them intraperitoneally with the various viral strains. To determine 50% lethal dose (LD_{50}) and mean time to death (MTD) for each of the Junin viral strains (table I), we inoculated groups of 15–20 guinea pigs with decimal dilutions of viral stocks and observed the animals for 60 days. The LD_{50} was calculated by probit analysis To determine organ viral titers and to examine histologically tissues from infected guinea pigs (table II, fig. 1), we inoculated groups of animals with 100 LD_{50} of each strain (5.0 $\log_{10}$ PFU for P3827, XJ44, and Candid No. 1). For each of these groups of guinea pigs, a single randomly chosen animal was killed by carbon dioxide, starting at 2- or 3-day intervals through day 21, starting on day 3 or 4 postinfection. With certain neurotropic strains of virus, some guinea pigs were paralyzed after day 21 postinfection. With these strains, after day 21, paralyzed guinea pigs were killed for the data presented in figure 1 and table II. These paralyzed guinea pigs are indicated with a P in figure 1 and table II.

Serological Assays

Indirect fluoresecent antibody (IFA) titers against Junin virus were determined according to the method of Peters et al. [15]. Plaque reduction neutralization (PRN) titers were performed on Vero cells by the method of Webb et al. [12].

Virus Determinations and Histological Preparations

Organ samples from killed guinea pigs were weighed and suspended in L-15 medium. These samples were triturated in tissue grinders and titrated for viable virus by plaque assay. Other samples were fixed on formalin and the sections stained with hematoxylin and eosin for light microscopy.

Classification of Human Syndrome

Human AHF is classified as mild, hemorrhagic, neurological, and mixed forms of the disease [9, 10]. The patients yielding the viral strains used in this study were classified at the time of their illness, but the form of the human disease remained unknown to those performing the experiments in guinea pigs.

Results

We infected 15–20 guinea pigs with low passage level Junin isolates to calculate the LD_{50} for each Junin strain tested in this animal model (table I). The range of calculated LD_{50} values for these strains was from <1 to >5.0 $\log_{10}$ PFU. With another group of 10–15 guinea pigs, we measured the MTD for each of these strains at a fixed 100 LD_{50}. The MTD varied from 14.5 (P3235) to 25 days or greater with other strains (P3684, P3827). Finally, in another experiment, 15–20 additional guinea pigs were infected with 5,000 PFU of the viral strains, and lethality and clinical signs were noted. At one end of the spectrum (P3235, P3790), the guinea pigs became lethargic and stopped eating on day 11 or 12, lost weight, and died before day 18. None of these animals was paralyzed. With other strains (P3684, P3827), guinea pigs appeared healthy until the 4th week after infection, when some of the animals became paralyzed and died. Candid No. 1 and XJ44 strains caused no signs of illness and no deaths.

Neither the MTD nor the clinical syndrome in the infected animals varied as a

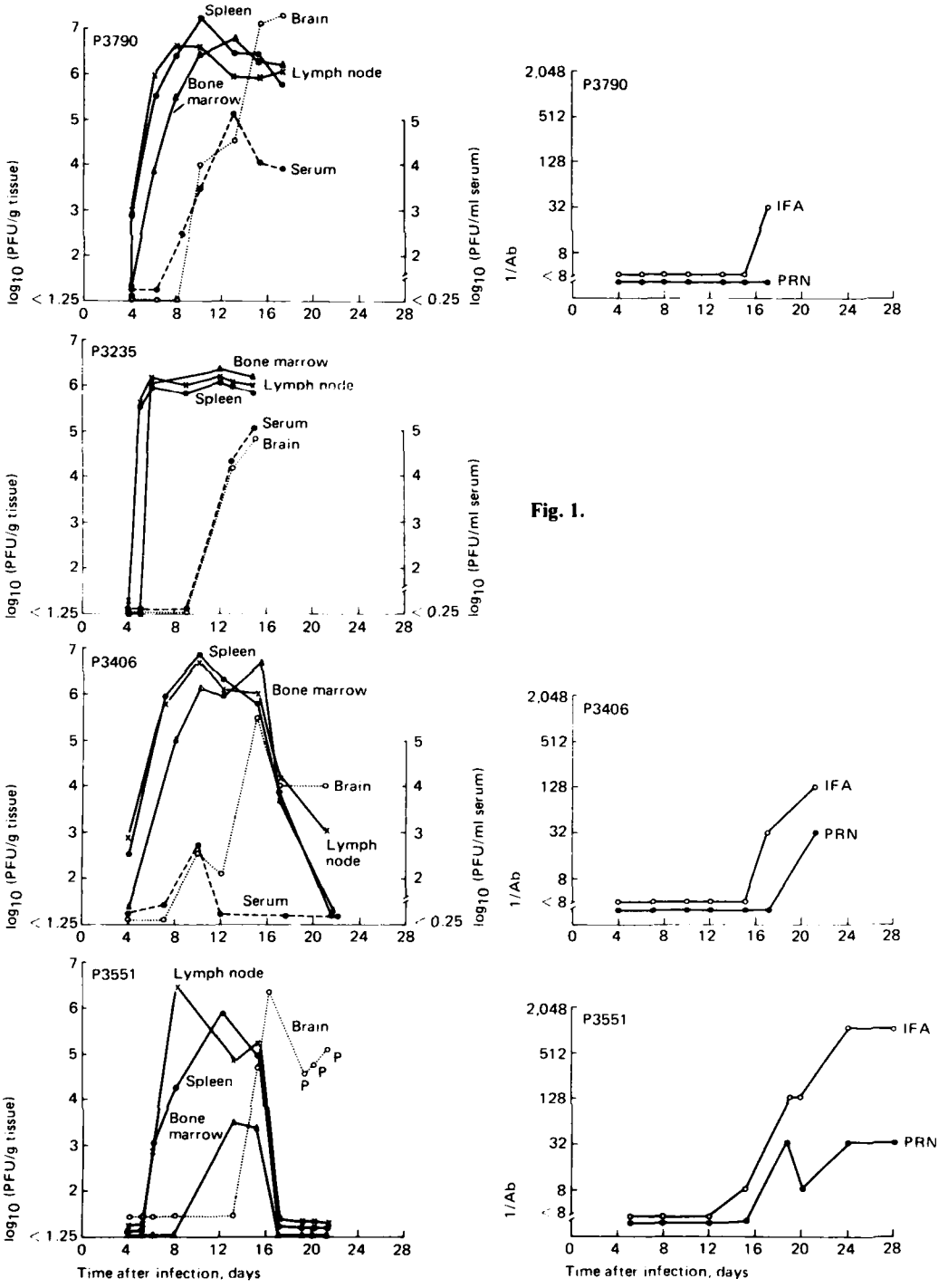
P3790
Spleen
Brain
Lymph node
Bone marrow
Serum
log10 (PFU/g tissue)
log10 (PFU/ml serum)
< 1.25
< 0.25
P3790
IFA
PRN
1/Ab
P3235
Bone marrow
Lymph node
Spleen
Serum
Brain
P3406
Spleen
Bone marrow
Brain
Lymph node
Serum
P3406
IFA
PRN
P3551
Lymph node
Brain
Spleen
Bone marrow
P
P3551
IFA
PRN
Time after infection, days
Time after infection, days

Fig. 1.

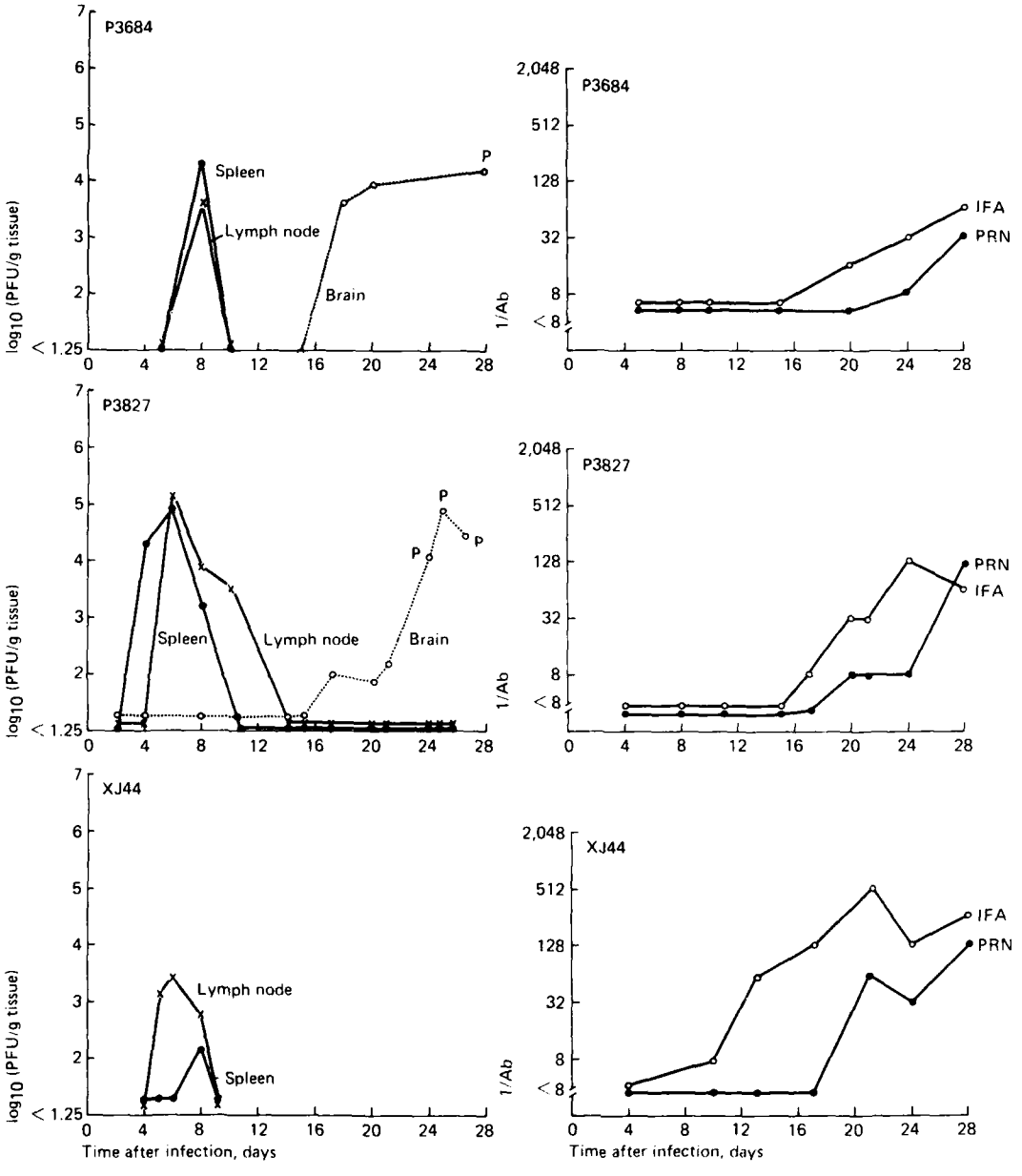

Fig. 1 cont. Organ viral titers and serum antibody titers of guinea pigs infected with different Junin viral strains.

Table II. Histological examination of tissues from guinea pigs infected with low-passage level Junin viral isolates[a].

Viral strain	Time after infection, days	Spleen	Lymph node	Bone marrow	Brain
P3790	4	0	0	0	0
	6	0	0	0	0
	8	+	+	0	0
	10	++	++	+	0
	13	++	++	+	0
	15	+++	+++	++++	+/0
	17	+++	++++	++	+
P3406	4	0	0	0	0
	7	0	0	0	0
	10	++	++	0	0
	12	++	++	+	0
	15	+++	+++	++++	+
	17	++	++	+++	+
	22	+++	++	0	+
P3235	4	0	0	0	0
	5	+	0	+	0
	7	0	+	+++	0
	9	++	++	0	+
	13	+++	+	++++	0
	15	++	++	++++	0
P3551	5	+	0	0	0
	8	++	++	0	+
	13	++	+	0	+
	17	ND	ND	ND	ND
	P19	+++	+++	+	+
	P20	++	+	++	+++
	P21	ND	ND	ND	ND
P3684	5	++	++	0	0
	7	+++	+++	0	0
	10	+++	+++	0	0
	15	+	+	0	0
	18	+	+	0	+
	20	+	+	0	+
	P27	+	+	0	+++
P3827	4	0	0	0	0
	6	ND	ND	ND	ND
	8	0	0	0	0
	11	0	0	0	+
	14	+	+	0	+++
	17	+	+	0	+++
	20	++	+	0	+++
	P24	+	+	0	+++
	P25	0	++	0	++++
	P26	++	+	0	++++

0 = No lesion(s); + = minimal lesion; ++ = mild lesion; +++ = moderate lesion; ++++ = severe lesion; ND = not done; P = guinea pigs paralyzed when killed.

[a] No significant lesions were found in tissues from guinea pigs infected with XJ44 or Candid No. 1 strains.

function of viral dose (data not shown). For instance, when the P3235 strain was inoculated in decimal dilutions from 5×10^5 to 5×10^{-2} PFU, all infected animals began to lose weight and died about the same time. The 50% infectious dose was the same as the LD_{50}. With the P3827 strain, increasing the inoculating dose had little effect. In groups receiving 10^6, 10^5, 10^4, or 10^3 PFU, we consistently observed paralysis in about 20% of the guinea pigs. All the guinea pigs inoculated with these doses were infected, since virtually all seroconverted, as determined by PRN assays (data not shown).

We killed guinea pigs infected with the different strains of Junin virus to determine organ viral and serum antibody titers and to examine the tissues histologically. For each viral strain, 12–15 guinea pigs were infected with 5,000 PFU, and a guinea pig was killed at each time point indicated in figure 1 and table II. The same time point in both figure 1 and table II represents the same guinea pig. Based on preliminary studies in our laboratory and on the studies of Elsner et al. [6] and Carballal et al. [8], the primary sites for virus isolation in Junin-infected guinea pigs are lymph nodes, spleen, bone marrow, brain, and serum. These were examined from each animal, and those sites positive for virus are shown in figure 1. Those sites negative at all time points are not shown. With two viral strains (P3790, P3235), we found hemorrhage of the gastric mucosa with pooling of blood in the stomach of moribund animals. None of the animals infected with the other strains showed gastric hemorrhaging, and none of the animals infected with any of the strains ever exhibited petechiae. We detected virus in the spleens and lymph nodes between days 4 and 6 postinfection with all viral strains except Candid No. 1. With this strain we

failed to detect virus in any of the organs at any of the time points. With the most highly virulent strains (as determined by percent mortality) the virus levels increased with time in spleen, lymph nodes, and, usually, bone marrow. In terminally ill guinea pigs, virus was detected in blood and brain. We termed this a visceral type illness. With other strains (usually less virulent, as measured by percent mortality), after 2–4 days of detectable virus in spleen and lymph nodes, virus could no longer be isolated from these organs. In the case of XJ44, there were no apparent signs of illness, and virus was not detectable in any of the organs through day 30. However, with other strains (P3827, P3551, P3684), virus appeared in the brains of the animals, usually in the 4th week after infection. These animals often exhibited progressive rear leg paralysis (indicated by P in figure 1 and table II) and had brain viral titers of a least 4.0 $\log_{10}$ PFU. We called this a neurologic illness. When we killed guinea pigs that were not paralyzed in the 4th week of infection with the P3827 strain, we found that these animals also had virus in the brains, but generally at a lower titer than their paralyzed counterparts (10^2 vs. 10^4 PFU/g; data not shown). Histological lesions in nonparalyzed animals were similar, although less severe than those seen in the paralyzed animals.

The predominant histological lesions found at different times after infection are shown in table II. There was a consistent lymphocytic depletion of spleen and lymph nodes with all Junin strains (except Candid No. I and XJ44) shortly after infectious virus was first detected in these organs. With some strains (P3684, P3827), this depletion was resolved, while with other strains (P3235, P3790, P3406), the lesions increased in severity, and we observed severe necrosis. The lymphoid depletion and necrosis was diffuse throughout both B and T cell dependent areas. With the highly visceral strains (P3235, P3790), there was also pancellular bone marrow depletion and necrosis. These two strains also produced severe, multifocal sites of gastric hemorrhage and necrosis. In contrast, strains producing neurologic disease typically induced only mild to moderate lymphocytic depletion in the spleen and lymph nodes, and there was no necrosis; the bone marrow remained normal. Brain lesions did not develop in guinea pigs infected with the visceral strains of virus. With the neurotropic virus, lesions usually appeared as mild encephalitis and polioencephalitis during the 3rd week after infection. This was most pronounced with the P3827-infected paralyzed guinea pigs which showed moderate to severe polioencephalitis, first appearing in the frontal cerebrum and, a few days later, in the medulla oblongata (data not shown). We recently examined spinal cords from animals infected with P3827 strain and found moderate to severe meningopoliomyelitis in the spinal cords as well as polioencephalitis in the brain. Not all guinea pigs inoculated with 5 $\log_{10}$ PFU of P3827 strain died (20% mortality), but the nonparalyzed animals we examined from days 21 to 30 showed virus in the brains and mild polioencephalitis; all animals had detectable PRN antibody titers.

Discussion

The mortality in guinea pigs after infection with viral isolates obtained from AHF patients varied from 20 to 100%, while the attenuated laboratory strains caused no illness or death. There was a wide range in MTD from 14.5 days (P3235) to greater than

25 days (P3684 and P3827). In contrast to that reported for Lassa viral isolates [16] the mortality was not significantly increased with the Junin isolates when strain 13 guinea pigs were infected (data not shown). Two patterns of disease became evident, a visceral and a neurologic form. The visceral form, typified by P3235 and P3790 strains, caused a rapidly fulminating illness with widely disseminated virus resulting in death between 2 and 3 weeks after inoculation. A common finding observed in this form of the disease was high viral titers in spleen, lymph node, and bone marrow. Virus was not detectable in the brain of these animals until a few days prior to death, and typically viral titers in the central nervous system (CNS) did not exceed those found in the blood. These infected guinea pigs were virtually never paralyzed. Histological examination showed alterations of spleen and lymph nodes indistinguishable from those reported for fatal human cases of AHF [17, 18]. These infected guinea pigs usually showed bone marrow necrosis, as is occasionally found in fatal human cases of AHF [10]. Pathology of the guinea pigs failed to pinpoint a single cause of death, but gastric hemorrhage was a contributing factor. Failure of bone marrow functions in these animals and compromised function of the lymph nodes and spleen perhaps were sufficient hematological and immunological injury to cause these strains to be uniformly lethal for their hosts in the 3rd week after infection.

At the opposite end of the spectrum, there was a different form of the disease typified by P3827 strain. These guinea pigs showed little evidence of the early clinical signs seen with the visceral forms, but a consistent proportion of animals showed paralysis 25 days after infection. There was low-level viral replication and minimal evidence of damage in the spleen and lymph nodes, but no viral replication or lesions were detectable in the bone marrow. The hallmark of this form of the disease was viral invasion of the brain by the 3rd week after infection which often manifested itself by rear leg paralysis. One might speculate that certain strains of moderate virulence that do not severely damage the spleen and lymph nodes, but in which sufficient viral replication occurs to seed the brain, may promulgate a type of disease exemplified as a late-onset paralysis. However, entry of virus into the brain did not seem to preclude recovery from the disease. We consistently found virus in the brains of guinea pigs infected with P3827, but only 20% of all the animals developed paralysis. Since antibody is thought to cross the blood-brain barrier very inefficiently [19], local synthesis of antibody or other immune mechanisms may be the critical events in the outcome of this disease form. We have been unsuccessful in obtaining cerebral spinal fluid from guinea pigs in sufficient quantity for antibody testing.

Pathology of the P3827 strain infected guinea pigs showed an unusual sparing of the lymphohematopoietic organs, whereas polioencephalitis was prevalent in the brain, and severe lesions were present along the spinal cord of guinea pigs that were examined [R. Kenyon, unpubl. observations]. There appeared to be a striking predilection for olfactory regions of the brain which was not observed with any other strain of Junin virus. In fact, we found detectable virus by plaque formation in olfactory lobes of guinea pigs 2–4 days prior to the time when we found virus in other parts of the brain [R. Kenyon, unpubl. observations]. This suggested the possibility that this virus gained entry to the

brain via the olfactory tracts. Experiments are in progress in which guinea pigs are infected via the respiratory route to compare the incidence of paralysis in animals infected by the peripheral versus the aerosol route.

Guinea pigs developing the visceral forms of AHF consistently showed severe bone marrow lesions. This has also been reported for guinea pigs infected with the prototype XJ strain of Junin [8] and for rhesus macaques infected with P3790, but not with P3406 [20]. Lesions in the bone marrow are not consistently found in human AHF [21], but, however, they are not a rare finding. These differences suggest a real variation in the pathogenic mechanisms of different strains of the virus, but may reflect the fact that guinea pigs are a more sensitive host to Junin virus than Primates.

There are two neurological aspects of AHF which are important to understanding the disease. With human infection there is often a neurological involvement during the acute phase of the disease, characterized by irritability, palmomental reflexes, abnormal gaits, and tremors [22]. Maiztegui et al. [23] showed that immune plasma therapy of human AHF reduced the mortality to 1%, but 11% of treated patients developed a late (2 or 3 weeks after hospital discharge) neurologic syndrome, dominated by vertigo, oculomotor palsies, and cerebellar signs [23, 24]. We cannot be sure that the early neurological involvement is caused by viral infection of the brain, but, based on earlier studies in our laboratory with the guinea pig model for AHF [25], a possible explanation for the late neurologic syndrome may be viral replication in the brain. It is possible that there is an immunopathological component to the neurological form of the disease, since an immune response, as measured by serum antibody, was often detectable in sick animals. We have been unable, however, to detect virus-specific cytolytic T cells in Junin-infected guinea pigs [26], so that component of the immune response could not be measured. Since we show here that only certain viral isolates are capable of inducing a neurological form of AHF in guinea pigs, we believe that further attention should be given to variations in Junin virus as determinants of pathogenesis in man.

Other available data from our laboratory suggest that antibody plays a pivotal role in termination of Junin viral infections [7, 11] by inactivating free virus and/or lysing infected cells. Although circulating antibody was not found before day 12 after infection with the attenuated XJ44 strain, we showed antibody-dependent, cell-mediated cytotoxicity (ADCC) by 6–8 days after infection [26], and shortly thereafter virus disappeared from the spleen and lymph nodes. We could not detect ADCC with P3235 strain, and organ viral titers continued to rise up to the time when the animals died at 14 days postinfection. With neurotropic strains, virus disappeared from lymph nodes and spleen a few days prior to the time when circulating antibody could be detected (we only tested for ADCC in XJ44 and P3235 infections). The virus later appeared in the brain. There appears to be a delicate balance between early viral replication and subsequent antibody synthesis. Some strains replicated rapidly and killed their host before an immune response occurred (P3235). Other viral strains replicated to a low level and then could no longer be detected (XJ44). Intermediate forms replicated, seeded the brain, and were eliminated (probably immunologically) from all organs but the CNS which led to a neurologic type of disease. Experimental intervention can upset

these balances. For instance, immunosuppressive drugs cause XJ44 strain to behave as a virulent strain [11], and correctly timed administration of immune serum can cause a P3235 infection to induce a neurologic type of disease [25].

Although arenaviruses are notorious for variations in their biologic properties based on passage history and emergence of variants [6, 27], we believe the differences seen here reflect valid genetically determined properties of the field strains tested. All were low-passage isolates, and the observed disease patterns were similar regardless of virus dose. Furthermore, three of the viruses were subjected to exhaustive plaque cloning, and each of the progeny clones bred true to parental virulence pattern (data not shown).

The clinical responses of rhesus monkeys infected with different strains of AHF correlated well with those of the AHF patients from which the strains were isolated [4]. The correlation did not appear as close in the guinea pig model. However, the human disease form is based on a single case, and the individual usually received immune plasma as well as supportive care. The disease form in rhesus monkeys was based on 3 or 4 animals. Neither of our most neurotropic strains (P3684 and P3827) has been tested yet in monkeys. Like humans, guinea pigs did exhibit different forms of AHF and reflected differences in pathogenic potentials of the strains. These differences appeared to be relatively stable. The strain differences should be remembered when considering some of the classical guinea pig pathogenesis data based solely on the prototype XJ strain. In fact, some of these studies state that the only reservation in the use of guinea pigs as a model for human AHF infection is the failure to show CNS involvement. Our data clearly show CNS involvement dependent on the strain of virus used. Perhaps, using the potentials available to us with the different forms of the guinea pig disease, we can more accurately model the different human disease forms to further advance treatment and supportive care.

References

1 Pirosky, I.; Zuccarini, J.; Molinelli, E.A.; Di Pietro; Barrera-Oro, J.G.; Martini, P.: Virosis hemorragica del noroeste bonaerense. Orientación méd. *8:* 171–180, 303–311 (1959).

2 Weissenbacher, M.C.; de Guerrero, L.B.; Boxaca, M.C.: Experimental biology and pathogenesis of Junin virus infection in animals and man. Bull. Wld Hlth Org. *52:* 507–515 (1975).

3 Weissenbacher, M.C.; Callelo, M.A.; Collas, D.J.; Golfa, H.; Frigero, M.J.: Argentine hemorrhagic fever: a primate model. Intervirology *11:* 363–367 (1979).

4 McKee, K.T.; Mahlandt, B.G.; Maiztegui, J.I.; Eddy, G.A.; Peters, C.J.: Experimental Argentine hemorrhagic fever in rhesus macaques: viral strain-dependent clinical response. J. infect. Dis. *152:* 218–221 (1985).

5 McKee, K.T.; Mahlandt, B.G.; Maiztegui, J.I.; Green, D.E.; Peters, C.J.: Virus-specific factors in experimental Argentine hemorrhagic fever in rhesus macaques. J. med. Virol. *22:* 99–111 (1987).

6 Elsner, B.; Boxaca, M.; Weissenbacher, M.; Guerrero, L.: Study of the experimental infection of guinea pigs with Junin virus. Medicina, Buenos Aires *36:* 197–201 (1976).

7 Peters, C.J.; Jahrling, P.B.; Liu, C.T.; Kenyon, R.H.; McKee, K.; Barrera-Oro, J.: Experimental studies of arenaviral hemorrhagic fevers. Curr. Top. Microbiol. Immunol. *134:* 5–68 (1987).

8 Carballal, G.; Cossio, P.M.; Laguens, R.P.; Ponzinibbio, C.; Oubina, J.R.; Meckert, P.C.; Rabinovich, A.; Arana, R.M.: Junin virus infection of guinea pigs: immunohistochemical and ultrastructural studies of hematopoietic tissue. J. infect. Dis. *143:* 7–14 (1981).

9 Maiztegui, J.I.: Clinical and epidemiological patterns of Argentine hemorrhagic fever. Bull. Wld Hlth Org. *52:* 567–575 (1975).

10 Peters, C.J.: Arenaviridae; in Belshe, Textbook of human virology, pp. 513–545 (PSG, Littleton 1984).
11 Kenyon, R.H.; Green, D.E.; Peters, C.J.: The effect of immunosuppression on experimental Argentine hemorrhagic fever in guinea pigs. J. Virol. *53:* 75–80 (1985).
12 Webb, P.A.; Johnson, K.M.; Mackenzie, R.B.: The measurement of specific antibodies in Bolivian hemorrhagic fever by neutralization of virus plaques. Proc. Soc. exp. Biol. Med. *130:* 1013–1019 (1969).
13 Centers for Disease Control: Classification of etiological agents on the basis of hazard; 4th ed. (Office of Biosafety, Centers for Disease Control, Atlanta 1976).
14 The Subcommittee on Arbovirus Laboratory Safety of the American Committee of Arthropod-Borne Viruses: Laboratory safety for arboviruses and certain other viruses of vertebrates. Am. J. trop. Med. Hyg. *29:* 1359–1381 (1981).
15 Peters, C.J.; Webb, P.A.; Johnson, K.M.: Measurement of antibodies to Machupo virus by the indirect fluorescent technique. Proc. Soc. exp. Biol. Med. *142:* 526–531 (1973).
16 Jahrling, P.B.; Smith, S.; Hesse, R.A.; Rhoderick, J.B.: Pathogenesis of Lassa virus infection in guinea pigs. Infect. Immunity *37:* 771–778 (1982).
17 Gonzalez, P.H.; Cossio, P.M.; Arana, R.M.; Maiztegui, J.I.; Laguens, R.P.: Pathology of lymphatic tissue in Argentine hemorrhagic fever. Archs Pathol. Lab. Med. *104:* 250–258 (1980).
18 Maiztegui, J.I.; Laguens, R.P.; Cossio, P.M.; Casanova, M.B.; de la Vega, M.T.; Ritacco, V.; Segal, A.; Fernandez, N.J.; Arana, R.M: Ultrastructural and immunohistochemical studies in five cases of Argentine hemorrhagic fever. J. infect. Dis. *132:* 35–43 (1975).
19 Griffin, D.E.; Hess, J.L.; Mokhtarian, F.: Entry of proteins and cells into the normal and virus-infected central nervous system; in Behan, Spreafico, Neuroimmunology, pp. 193–206 (Raven Press, New York 1984).
20 Green, D.E.; Mahlandt, B.G.; McKee, K.T., Jr.: Experimental Argentine hemorrhagic fever in rhesus macques: virus-specific variations in pathology. J. med. Virol. *22:* 113–133 (1987).
21 Ponzinibbio, C.; Gonzalez, P.H.; Maiztegui, J. I.; Laguens, R.P.: Estudio morfologico de la medula osea humana en fiebre hemorragica Argentina. Medicina, Buenos Aires *39:* 441–446 (1979).
22 Biquard, C.; Figini, M.A.; Monteverde, D.A.: Estudio neurologico de 120 casos de fiebre hemorragica Argentina. Pensa méd. Argentina *56:* 605–614 (1969).
23 Maiztegui, J.I.; Fernandez, N.J.; de Damilano, A.J.: Efficacy of immune plasma treatment of Argentine hemorrhagic fever and association between treatment and a late neurological syndrome. Lancet *ii:* 1216–1217 (1979).
24 Melcon, M.O.; Herskovits, E.: Complicaciones neurologicas tardias de la fiebre hemorragica Argentina. Medicina, Buenos Aires *41:* 137–145 (1981).
25 Kenyon, R.H.; Green, D.E.; Eddy, G.A.; Peters, C.J.: Treatment of Junin virus-infected guinea pigs with immune serum: development of late neurological disease. J. med. Virol. *20:* 207–218 (1986).
26 Kenyon, R.H.; Peters, C.J.: Cytolysis of Junin virus infected target cells by immune guinea pig spleen cells. Microbial Pathogen. *1:* 453–464 (1986).
27 Ahmed, R.; Salmi, A.; Butler, L.T.; Chiller, J.M.; Oldstone, M.B.A.: Selection of genetic variants of lymphocytic choriomeningitis virus in spleens of persistently infected mice: role in suppression of cytotoxic T lymphocyte response and viral persistence. J. exp. Med. *60:* 521–540 (1984).